Immunohistochemical detection of double-infected cell bodies

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Method Article

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Abstract

Neural circuitry in the lumbar spinal cord governs two principal features of locomotion, rhythm and pattern, which reflect intra- and interlimb movement. These features are functionally organized into a hierarchy that precisely controls stepping in a stereotypic, speed-dependent fashion. Here, we show that a specific component of the locomotor pattern can be independently manipulated. Silencing spinal L2 interneurons that project to L5 selectively disrupts hindlimb alternation allowing a continuum of walking-to-hopping to emerge from the otherwise intact network. This perturbation, which was is independent of speed and occurred occurs spontaneously with each step, did does not disrupt multi-joint movements or forelimb alternation, nor did does it translate to a non-weight bearing locomotor activity. Both the underlying rhythm and the usual relationship between speed and spatiotemporal characteristics of stepping persisted. These data illustrate that hindlimb alternation can be manipulated independently from other core features of stepping, revealing a striking freedom in an otherwise precisely-controlled system.

Reagents

Cross-sections of spinal sections mounted to charged glass slides Sodium citrate buffer \(pH 6) 3% hydrogen peroxide DAB IHC Select kit \(DAB500, Millipore) Rabbit anti-GFP primary antibody \(abcam ab290) 0.1 M PBS Bovine serum albumin Normal goat serum 75% ethanol 95% ethanol 100% ethanol xylenes Permount

Equipment

Humidor chamber Pap pens Slide warmer Shaker Trays \(e.g. lid of 24 well cell culture dish)

Procedure

1.Using a cryostat, cut the lumbar segments where the double-infected cell bodies reside \(e.g. L2 for L2-L5 interneurons). Cut the tissue at 30 µm in sets of 5, mounting the sequential cross-sections across each slide until there are five cross-sections per slide \(e.g. first section cut mounted to slide A1, second section – B1, third section, C1, etc). Store slides at -20ºC. 2.Turn slide warmer on and set to 67°C. When at temperature, warm one complete set of slides \(e.g. slides A1, A2, A3, etc) for 30 minutes. 3.During this stage, prepare for the next step: heat-induced epitope retrieval. Bring 500 ml of sodium citrate buffer \(pH 6.0) to a rolling boil \(95-100°C) on a hot plate. Add a magnetic spin vane to provide gentle mixing. 4.After the slides have warmed, load them into a heat-resistant housing chamber and lower them into the boiling citrate solution. Incubate for 40 minutes. 5.Remove slides and allow them to cool to room temperature \(approximately 5 minutes). 6.Outline the sections with a hydrophobic slide marker and transfer them to a humidor chamber. All of the following incubations will be performed with gentle shaking at room temperature unless otherwise noted. 7.Rinse the sections with 0.1 M PBS \(pH 7.4) for two, 5 minute washes. 8.Treat the sections with 3% hydrogen peroxide for 10 minutes. 9.Quench the

reaction with the DAB rinse buffer provided in the DAB IHC Select kit from Millipore \(DAB500). Perform three, 5 minute washes. 10.Apply the DAB IHC Select kit blocking solution, incubate for 10 minutes. 11.Perform three, 5 minute washes with the DAB rinse buffer. 12.During the final rinse, prepare the primary antibody solution \(rabbit anti-GFP at 1:5,000 in a solution of 0.1 M PBS, 0.5% bovine serum albumin, and 5% normal goat serum). 13.Incubate the primary antibody overnight at 4°C with gentle shaking. 14.The following day, perform three, 5 minute washes with the DAB rinse buffer. 15.Apply the DAB IHC Select kit secondary antibody solution, incubate for 10 minutes. 16.Perform three, 5 minute washes with the DAB rinse buffer. 17.Apply the DAB IHC Select kit streptavidin HRP solution, incubate for 10 minutes. 18.Perform three, 5 minute washes with the DAB rinse buffer. 19.During the final rinse, prepare the chromagen reaction solution. Create a cocktail of the provided DAB-A and DAB-B solutions per the instructions provided in the DAB500 kit. 20.After aspirating off the final rinse, transfer the slides to separate trays \(e.g. the lids from 24-well cell culture dishes). Apply the DAB solution and incubate for 10 minutes. Increase the speed of the shaker such that the solution is vigorously mixing. Rotate the trays clockwise every two minutes to prevent settling of DAB precipitate onto the underlying cross-sections. 21.Immediately stop the reaction by decanting DAB rinse buffer into the trays, raising the DAB solution away from the cross-sections. Let shake for another five minutes at a lower speed. 22.Decant the DAB-rinse buffer solution in a liquid biohazard waste container and apply fresh DAB rinse buffer solution. Shake at moderate speed for approximately 30 minutes. 23.Remove slides from the trays and dehydrate in an increasing ethanol gradient of 75%, 95%, and 100% \(each three minutes, respectively). Thereafter, incubate the slides in xylenes for three minutes. 24.Coverslip with Permount and allow to dry at room temperature overnight before imaging.